Research Note: A nondestructive method for cleaning gastropod radulae from frozen, alcohol-fixed, or dried material

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Abstract: A new method for cleaning tissue from gastropod radulae is presented that is non-destructive compared to the methods presently in use. Present methods are time sensitive because the caustic solutions used can damage radula. This new method uses a detergent and Proteinase K enzyme which are not time sensitive. This method will work with frozen, alcohol-fixed, or dried material but not with formalin-fixed material. In addition, the tissue supernatant can be used for subsequent standard DNA-extraction techniques.

Key words: gastropod, snail, radula, cleaning method, preparation

The present methods for cleaning gastropod radulae for viewing use either sodium hypochlorite (NaOCl) or potassium hydroxide (KOH). Because of the caustic nature of these chemicals, careful monitoring of the specimen is required to avoid damaged or dissolved radulae. An alternative method routinely used to digest tissue for DNA extraction (Sambrook *et al.*, 1989) has proven to be as effective in cleaning radulae and avoids the likelihood of unwanted erosion of the specimens. This method is effective for preparing radulae from frozen, alcohol-fixed, newly dried, and from dried specimens collected over 30 years ago. This method has not proven to be useful for cleaning radulae from specimens preserved in formalin because of the protein cross-linking that occurs with this method of fixation.

MATERIALS

The necessary equipment and stock solutions required are listed in Table 1. In addition, the following lab equipment is needed: dissecting microscope, microforceps, scalpel, and pipettes. Additional useful tools include: insect pins (very good probes) and sharpened applicator sticks (excellent tools for manipulating the radula during mounting). Cleaned radulae for scanning electron microscopy (Fig. 1) were sputter-coated with gold-palladium and scanned on a Hitachi S-2500 scanning electron microscope at the University of Alabama.

THE METHOD

With freshly frozen material the gastropod is generally partially extended so that it is a simple matter to extract the animal from its shell by grasping the foot and body behind the head and gently pulling. Typically in alcohol-fixed and/or dried material, the animal has withdrawn into the shell. Removal of the snail body from the shell can be accomplished by carefully cracking the shell. After separating the head from the rest of the animal, rinse the head with water to remove any extraneous material that might have adhered to it (*i. e.* shell fragments). Place each specimen in an individual 1.5 ml microcentrifuge tube and add 500 µl NET buffer and 10 µl Proteinase K (Table 1). Close the tube and tape it to the mixer platform, and place the mixer an incubator at 37°C. With fresh or alcohol-fixed material, cleaning should take about 2-3 hr. Dried tissue specimens can require 2-4 d, depending on the amount of starting material. For dry material it may be necessary to replace the NET buffer and Proteinase K after 24-48 hr. Once cleaning is complete, as determined by examination under a dissecting microscope, remove the radula and rinse in several changes of deionized water to removed all traces of the NET buffer. The remaining supernatant can be used to obtain DNA using DNA standard phenol/chloroform extraction techniques. The radula is then stored in 25% ethanol until ready for mounting. At this point the clean radula can be mounted by a procedure appropriate for the type of visualization to be used. Some cleaned radulae have

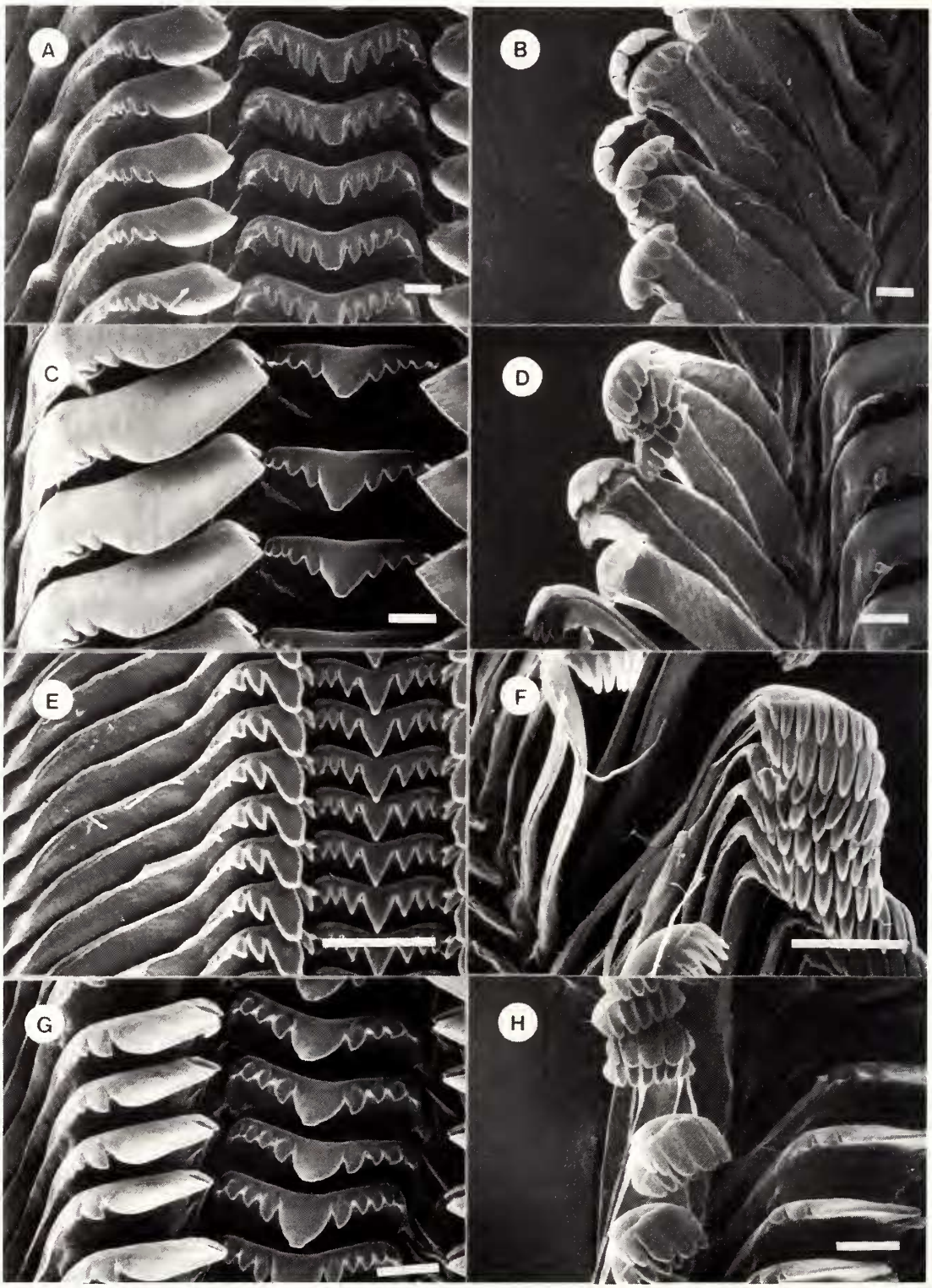

Fig. 1. Examples of radulae cleaned by the described method. A-B. *Io fluvialis* (Say, 1825). C-D. *Leptoxis (Athearnia) crassa anthonyi* (Redfield. 1854). E-F. *Terebia granifera* (Lamarck, 1822). G-H. *Gyrotoma pyramidatum* Shuttleworth, 1845. The radulae were prepared from the following types of tissue: fresh frozen (A-B); alcohol fixed (C-D); alcohol fixed and dried; (E-F); 30-year old unfixed dried material (G-H). Figs. A, C, E, and G show the central and left lateral tooth; B, D, and H show the left pair of inner and outer marginal teeth; and F shows the right pair of inner and outer marginal teeth. Scale bars = 50 μm.

Table 1. Equipment and stock solutions.

Incubator
Platform mixer
1.5 ml microcentrifuge tubes
NET buffer
1 ml Tris pH 8.0
2 ml 0.5 M ethylene diamine tetraacetic acid (EDTA)
1ml 5 M NaCl
20 ml 10% sodium dodecyl sulfate (SDS) (Sigma-Aldrich Chemical Company no. L4522)
76 ml deionized water
Proteinase K stock*
20 mg Proteinase K* (Sigma-Aldrich Chemical Company no. P4914)
1 ml deionized water
* store at -20 °C

been stored in 25% ethanol for up to 6 mo before mounting and no degradation was observed.

The radula and the radular ribbon do not appear to be affected by the enzymatic action of Proteinase K as evidenced by scanning electron micrographs (Fig. 1). Therefore in this cleaning method careful monitoring is not necessary. An additional benefit of this procedure is that the radular ribbon from dried material is rehydrated and becomes flexible enough to undergo the rigors of mounting.

LITERATURE CITED

Sambrook, J., E. F. Fritch, and T. Maniatis. 1989. *Molecular Cloning, a Laboratory Manual, 2nd ed.* Cold Spring Harbor Laboratory Press, New York. 3 vols.

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